Susceptibility of Fruit from Diverse Apple and Crabapple Germplasm to Attack from Apple Maggot (Diptera: Tephritidae)

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ABSTRACT Apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), is a pest of major concern to apple, *Malus* × *domestica* (Borkh.) production in eastern North America. Host plant resistance to apple maggot among apple germplasm has been previously evaluated among a small number of exotic *Malus* accessions and domestic hybrid selections. However, a large number of exotic accessions housed in USDA collections have never been evaluated for their susceptibility to apple pests. Additionally, previous reports of resistance need to be confirmed under both field conditions and with more rigorous laboratory evaluations. Thus, studies were conducted to evaluate the susceptibility of a number of *Malus* accessions housed at the USDA Plant Genetic Resources Unit "core" collection. Contrary to earlier published reports, these results suggest that some selections previously described as "resistant" are in fact susceptible to both oviposition damage and larval feeding damage by apple maggot. One domestic, disease-resistant apple accession, 'E36-7' is resistant to survival of apple maggot larvae except when the fruit is nearly ripe in late fall. This is the first report of an apple cultivar that is confirmed to be resistant to larval feeding of apple maggot. Although adults can successfully oviposit on all accessions examined, larval survival was zero in a number of small-fruited crabapple accessions classified as resistant in previous studies and also in two accessions, *Malus tschonoskii* (Maxim) C. K. Schneid. and *M. spectabilis* (Aiton) Borkh., that have not been previously evaluated.

KEY WORDS *Rhagoletis pomonella*, apple, germplasm, host plant resistance

The apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), is indigenous to eastern North America and originally infested fruit of various species of hawthorn (*Crataegus* spp.). The first apple maggot infestation in cultivated apples, *Malus pumila* (Mill.), was reported in 1866 in New York, ≈200 yr after the fruit was introduced into the United States from Europe (Illingworth 1912). Currently, sympatric populations of *R. pomonella* infesting either apples or hawthorn are found throughout northeastern North America (Bush 1969, Feder et al. 1994). The apple maggot is one of the most serious pests of apples in eastern production areas, and because larvae feed internally in the fruit, there is little to no tolerance for infestation. Consequently, eastern apple growers have traditionally applied multiple sprays of insecticides during July and August to control this pest (Reissig 1988) and efficacious alternative control tactics for this pest are generally lacking.

Although a good deal of research has been done to develop alternative management approaches for this pest, little research has investigated the selection and development of resistant apple cultivars, which could be used in integrated pest management programs to reduce insecticide use. All currently grown commercial apples are infested by the apple maggot, although differences in oviposition and larval survival are known to occur among different cultivars. Dean and Chapman (1973) observed that females preferred to oviposit in early ripening, subacid varieties of apples and larval survival was greatest in early maturing or soft-fleshed cultivars. Rull and Prokopy (2004) confirmed that laboratory oviposition behavior was affected by fruit maturity, and they showed that tree visitation rates in the field can vary by apple genotype.

Little recent work has been done to compare differences in apple maggot oviposition preference and subsequent larval survival in different *Malus* species and various apple breeding lines. Goonewardene et al. (1975, 1979) and Goonewardene and Howard (1989) screened disease-resistant apple clones by using no-choice laboratory tests. They classified some of these lines as resistant to apple maggot (USDA 2006), although females oviposited in most of the fruit and larvae subsequently completed their development in apples from all but one of the clones. Neilson (1967) conducted laboratory studies showing that caged apple maggot females readily oviposited in many varieties of crabapples that were rarely infested in the field, although no larvae survived in fruit of the Sibe-

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rian crab [*Malus baccata* (L.) Borkh], *M. sikkimensis* (Hook) Kochae—now revised to *M. sikkimensis* (Werz.) Koehne ex. C. K. Schneid.—*M. toringoides* Huges—now revised to *M. bhutanica* (W. W. Sm.) J. B. Phipps—'Henry F. DuPont', 'Henrietta Crosby', or 'Almey'. Pree (1977) identified an additional cultivar, 'Morden 455′, that was also resistant to larval development.

Reissig et al. (1990) reported that oviposition and larval survival of the apple maggot varied significantly among fruit from 25 crab apple species and clones evaluated in field and laboratory studies. Although in laboratory tests females oviposited and eggs hatched in all 25 crabapples evaluated, larvae did not survive in Henry F. DuPont, 'Frettingham', 'Fuji', 'Sparkler', '*M. hupehensis* (Pamp.) Rehder, or *M.* × *zumi* (Matsum.) Rehder 'Calocarpa.' Larval mortality was very high in fruit from *M. yunnanensis* (Franch) C. K. Schneid. 'Vilmorin', 'NA 40298′, Henrietta Crosby, 'Golden Gem', Almey, *M. baccata* L. (Borkh), and *M. sikkemensis* (Hood.) Koehne [now revised to *M. sikkimensis* (Werz.) Koehne ex. C. K. Schneid.]. Beyond these preliminary evaluations, the majority of genetic diversity found within *Malus* has never been evaluated for susceptibility to apple maggot.

Thus, in 2005–2006, studies were conducted to evaluate the apple maggot susceptibility of fruit from a number of *Malus* accessions housed in a collection with representation of ≈90% of the known genetic diversity of *Malus.* These studies are the first reports of no-choice and choice assays for oviposition preference and survival of larvae in these apple and crabapple accessions. These laboratory assays, conducted with an in-house apple maggot colony, were complemented by field screening of many of the same accessions for susceptibility to attack from a wild apple maggot population under conditions comparable with those in a commercial apple orchard, with the exception of insecticide applications.

Materials and Methods

Apple Maggot Colony. All flies used in laboratory assays were derived from a colony that has been reared continuously in the laboratory for ≈30 yr and housed in the Department of Entomology at the New York Agricultural Experiment Station in Geneva, NY. The colony was maintained as follows: Adult flies were fed on an artificial diet consisting of sucrose, a vitamin mixture (MP #904654, MP Biomedicals, LLC, Solon, OH), casein hydrolysate (Nz-Amine A, MP #101290, MP Biomedicals, LLC), and salt (USP XIII No. 2 salt mixture, MP #902845, MP Biomedicals, LLC) (Neilson and McAllan 1965). To preserve behavior similar to populations of wild flies, this colony has been reared on 'Delicious' apples. Adults were confined in screened, wood-framed rearing cages (Neilson 1965), and flies mated primarily on fruit as they normally do in nature. After mating, gravid females oviposited in the fruit and larvae developed in apples until the mature larvae leave the fruit and were later collected as pupae as described by Neilson (1965). Pupae were then placed into rearing cages where adults emerged to complete their life cycle. All larvae and adults were maintained in walk-in growth chambers set to a photoperiod of 16:8 (L:D) h. Emergence cages with adult flies <1 wk old were stored at 24°C and 35% RH. Cages with flies older than 1 wk were stored at 23°C and 50% RH. Apple fruit containing developing larvae were enclosed in cloth covered pupation funnels and stored at 27°C and 55% RH.

Laboratory Oviposition Assays, 2005–2006. Both no-choice and choice adult oviposition assays were conducted in 2005–2006 by using fruit collected from the USDA Plant Genetic Resources Unit (PGRU) collections housed in Geneva, NY. A listing of all accessions evaluated, with block and tree locations, is given in Table 1. With the exception of two accessions housed at other sites, key accessions used for laboratory studies were housed in the "core" *Malus* germplasm collection. This core collection (Grauke et al. 1995, Kresovich et al. 1995) includes 206 diverse *Malus* accessions from the total collection of 2,438 clonal accessions as described by Forsline (1996). It was planted in 1991 in a replicated block on the Darrow Farm of the New York State Agricultural Experiment Station and consists of the various *Malus* accessions grafted onto M 27 rootstocks. The block is located adjacent to an abandoned apple orchard and various plantings of apricots, plums, and strawberries (Fig. 1). The collection was not treated with any insecticide sprays during the 2005 or 2006 growing seasons, but it did receive standard treatments of fungicides and groundcover herbicides. Fruit from one accession were derived from a *M. sieversii* (Lebed.) seedling tree housed at the McCarthy farm at PGRU. It is derived from a seed originally collected in Kazakhstan at site 11 as described by Forsline et al. (2003).

Fruit collected for bioassays were kept in a cold storage room (≈5 ± 1°C) until they were used in a bioassay. Fruit were kept in storage for 1–10 d before use in all laboratory assays. For no-choice studies, single fruit were suspended from the bottom of overturned 0.946-liter clear round plastic cups. Fruit were suspended by the stem with florist wire, which was hung from the container through a small pinhole, where it was attached to a large paper clip on the outside of the cup. Female adults used for laboratory assays were taken from mating chambers where they had been held for at least 2 wk. Two mated female flies from the Geneva apple maggot colony (reared as described previously) were placed into each plastic cup chamber after 48-h exposure to Delicious apples, which helps to reduce their very strong oviposition drive and enhances their discrimination in host selection (Prokopy 1972).

Oviposition chambers were each provisioned with a food source consisting of a 3-cm cotton wick saturated with a 10% sucrose solution, enclosed in an uncapped 3-ml glass vial. Vials were placed in an ≈1-cm hole cut into the side of each cup. For choice studies the same protocol was followed except that an additional fruit (*M.* × *domestica* 'Marshall McIntosh') was added as a comparative standard 'McIntosh' is one

Table 1. ***Malus*** **accessions used for field assessments, laboratory assays, or both are listed with the corresponding identification (PI) number as used in the USDA GRIN database[a] with field locations of trees at the core germplasm collection, and other** ***Malus*** **collections sites, (PGRU-Core),[b] Geneva, NY, 2005–2006**

Species	Cultivar	GRIN PI no.	PGRU-core block, row-tree	Other PGRU sites (block) row-tree
M. × *domestica* (Borkh.)	Delicious	589841	6-15, 9-51, 10-60	
M. × *domestica* (Borkh.)	Liberty	588943	1-53, 6-1, 8-29, 10-65	
M. hybrid	'PRI 1293-102'	590074		(T-1)38-35
M. hybrid	PRI 1312-6	590079	3-28, 6-49, 8-17, 11-49	
M. hybrid	'E11-24'	589571	1-20, 5-24	
M. hybrid	E14-32	589572	6-64, 8-49, 12-28	
M. hybrid	'E7-47'	590069	1-25, 5-30, 9-6, 11-38	
M. hybrid	E7-54	590070	1-26, 4-15, 10-42	
M. hybrid	E29-56	590071	1-22, 5-65, 7-61, 10-11	
M. hybrid	'E31-10'	590072	1-23, 6-22, 7-27	
M. hybrid	E36-7	589570	1-24, 4-57, 8-45	
M. hupehensis (Pamp.) Rehder		594098	2-18, 6-59, 7-60, 11-15	
M. sieversii (Ledeb.) M. Roem.	Seedling	GMAL 4304.e		(K-1)20-51
M. sikkimensis (Wenz.) Koehne ex C. K. Schneid		589390	4-11, 8-19, 11-26	
M. spectabilis (Aiton) Borkh.		594100	2-57, 6-13, 7-48, 10-52	
M. bhutanica (W. W. Sm.) J. B. Phipps	Macrocarpa	588930	2-64, 6-19, 7-38	
M. tschonoskii (Maxim) C. K. Schneid.		589395	3-1, 9-19	
M. yunnanensis (Franch.) C. K. Schneid.	Vilmorin	271831	3-14, 5-40, 7-19, 12-49	
M. × *zumi* (Matsum.) Rehder	Calocarpa	589840	3-11, 4-63, 8-42, 11-31	

[a] Available online at http://www.ars-grin.gov/npgs/index.html.
[b] Plant Genetic Resources Unit, USDA–ARS, Cornell University 630 W. North St., Geneva, NY 14456.

of the oldest, most widely grown cultivars in the northeastern United States. Both fruit were suspended inside a larger rectangular, capped, 1.89-liter-deep dish plastic container (GladWare Glad Products Company, Oakland, CA). For both no-choice and choice studies, flies were removed from the plastic oviposition chambers after 48 h of exposure to fruit. Fruit height and diameter were recorded, and each fruit was carefully examined under a stereomicroscope for the presence of oviposition scars. Five to 10 fruit (or fruit pairs for choice studies) replicates were examined for every *Malus* accession in each assay.

For no-choice assays, the number of oviposition scars per fruit were analyzed by accession using a

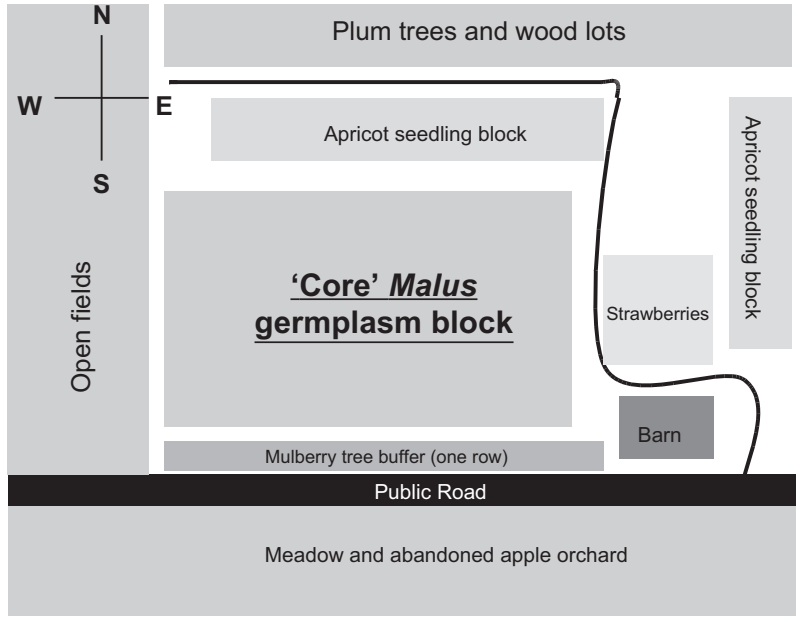

Fig. 1. Core germplasm block used for field evaluations of host plant resistance to insect pests among numerous *Malus* accessions, and the surrounding landscape, USDA–PGRU, Geneva, NY.

Table 2. Mean fruit diameter and mean ± SEM number of female oviposition scars on fruit collected during the mid-season (25 July) and near harvest (20 Sept.) after 48-h exposure to single fruit in a no-choice assay with various *Malus* accessions in the laboratory, 2005

Date[a]	Accession	n[b]	Avg. fruit diam. (mm)	Punctures per fruit[c]	Punctures per sq. mm of fruit surface area[c]
25 July	E36-7	9	49.5	14.44 (4.32)a	0.049 (0.015)a
	Delicious	10	53.9	21.80 (5.13)a	0.070 (0.017)ab
	Liberty	10	49.7	23.00 (7.45)a	0.082 (0.027)ab
	E7-54	10	52.0	26.40 (7.99)a	0.091 (0.027)ab
	E7-47	8	46.2	25.25 (6.66)a	0.096 (0.026)abc
	PRI 1312-6	10	59.3	32.80 (9.76)a	0.096 (0.029)abc
	E31-10	10	56.8	32.80 (11.86)a	0.105 (0.038)abc
	E11-24	10	47.1	28.70 (5.26)a	0.106 (0.019)abc
	E29-56	8	52.6	38.00 (4.45)a	0.127 (0.016)abc
	PRI 1293-102	8	44.5	34.13 (11.00)a	0.133 (0.042)abc
	Marshall MacIntosh	9	49.0	39.33 (7.86)a	0.143 (0.030)bc
	E14-32	10	46.7	47.00 (8.96)a	0.175 (0.033)cd
	M. sieversii 'GMAL 4304.e'	9	29.6	40.78 (7.70)a	0.227 (0.043)d
20 Sept.	PRI 1312-6	10	80.3	13.90 (2.50)a	0.030 (0.005)a
	E29-56	10	66.3	13.80 (4.07)a	0.036 (0.010)ab
	E36-7	10	66.8	18.90 (3.05)a	0.050 (0.009)abc
	E7-54	10	69.4	22.70 (3.05)a	0.056 (0.009)bc
	E14-32	10	65.6	21.20 (4.10)a	0.037 (0.012)abc
	Marshall MacIntosh	10	70.9	28.80 (4.83)a	0.072 (0.012)c

[a] Date of fruit harvest.
[b] Number of fruit examined.
[c] Means within each column and date grouping followed by the same letter are not significantly different ($P < 0.05$; Fisher LSD).

one-way analysis of variance (ANOVA) on ln-transformed data (SAS Institute 2002). Means were separated using Fisher's protected least significant difference (LSD) test ($P < 0.05$). Additionally, oviposition punctures per square millimeter of fruit surface area were calculated to correct for differences in fruit size, and the raw data were analyzed by accession using a one-way ANOVA (SAS Institute 2002). Means were separated using Fisher's protected LSD test ($P < 0.05$). For choice assays, the number of oviposition scars on fruit of each accession was compared with the number of scars on fruit from Marshall McIntosh by using a paired t-test ($P < 0.05$) of ln-tranformed data (Minitab, Inc. 2005). In 2005, both assays were conducted twice (mid-season assessments initiated on 25 July, near harvest assessments initiated on 20 September). In 2006, both assays were conducted three times (mid-season assessments initiated 17 July, late season assessments initiated on 31 August, near harvest assessments initiated on 14 September). Although fruit maturity states at these different stages varied somewhat between cultivars (dates are given in Table 9), these discrete intervals were chosen to observe response to the different cultivars at various times of the growing season.

Larval Survival, 2005–2006. Survival of larvae was assessed using the same fruit used for the adult no-choice oviposition assays. The fruit were thoroughly examined for oviposition and the number of punctures was recorded. Fruit within each accession group were pooled into two cohorts of five fruit each due to space limitations (i.e., rather than trying to observe larval emergence from each fruit). Fruit were placed in rectangular plastic tubs (≈40 by 60 by 15 cm in depth) on metal mesh screening (hole size of ≈1 by 1 cm) with holes large enough for emerging maggots to fall through. The screening was placed so that fruit were held several centimeters above the bottom of the plastic container. The containers were then partially filled with water to a depth of ≈2–3 cm. Plastic containers were capped and placed in a walk-in growth chamber set to 23°C and 50% RH, and photoperiod 16:8 (L:D) h. Larval emergence was evaluated by periodic counting and removal of apple maggot larvae that fell from fruit into the water. Containers were checked twice weekly for 6–8 wk for emergence. Water was replenished in the plastic containers as needed. When emergence was complete, the total number of larvae was tallied for each cohort of fruit. Percentage of larval survival was calculated by dividing the number of emerged larvae by the total number of oviposition scars originally present on all of the fruit within each cohort. For this calculation, it was assumed that every oviposition scar contained a viable egg. Percentage of survival was analyzed by accession using a one-way ANOVA on arcsine-transformed data (SAS Institute 2002). Means were separated using Fisher's protected LSD test ($P < 0.05$).

Harvest Assessment of Apple Maggot Damage in the Field, 2006. Fruit from a number of *Malus* accessions were examined for apple maggot feeding damage via on-tree visual inspection in the field from 2 to 6 October 2006 at the USDA–PGRU core *Malus* germplasm collection (described above). Optimally, a total of 50 fruit per tree from each accession were visually examined, although some trees had less (a minimum of 20 fruit were necessary for the tree's damage rating to be counted). All accessions had two to four replicate trees within the block. Because early season apple maggot feeding damage can be difficult to differentiate from damage caused by other catfacing insect species, fruit were cut open in some cases to confirm the presence of the apple maggot's distinctive larval tunneling damage. Percentage of fruit damage was

Table 3. Mid-season (fruit picked 25 July) and near harvest (fruit picked 27 September) adult oviposition preferences after 48 h of exposure, given a choice between a fruit from a given *Malus* accession and a Marshall MacIntosh control in the laboratory, 2005

Accession	n^a	Mid-season preference[b]	n	Near harvest preference[b]
Marshall MacIntosh	10	N		N
Delicious	10	A*		
Liberty	10	N (A*)		
E36-7	10	N		N
E7-54	10	N		N
E7-47	10	N		
E31-10	10	A*		
E11-24	10	N		
E29-56	10	N		N
E14-32	10	N		A*
PRI 1293-102	10	N		
PRI 1312-6	10	N		A*
M. sieversii GMAL 4304.e	10	N		

[a] Number of fruit pairs examined.

[b] N indicates no significant preference for either fruit; A indicates a significant preference for the experimental fruit, as determined by a paired *t*-test ($P < 0.05$) with ln-transformed data.

*Indicates the preference was also significant when puncture data were adjusted for fruit size.

calculated for each tree by dividing the number of fruit with visible apple maggot larval feeding tunnels by the total number of fruit examined for each tree replicate. Percentage of damage was analyzed by accession using a one-way ANOVA on arcsine-transformed data (SAS Institute 2002). Means were separated using Fisher's protected LSD test ($P < 0.05$).

Results

Laboratory Oviposition Assays, 2005–2006. There was no difference in the number of apple maggot oviposition punctures per fruit by accession in either mid-season ($F = 1.23$; df = 12, 108; $P = 0.2691$) or near harvest ($F = 2.24$; df = 5, 54; $P = 0.0630$) no-choice assays conducted in 2005 (Table 2). When oviposition was corrected for fruit surface area, significant oviposition differences were observed in both mid-season ($F = 2.55$; df = 12, 108; $P = 0.0050$) and near harvest ($F = 2.52$; df = 5, 54; $P = 0.040$) assays, although moderate oviposition damage was present on all accessions (Table 2). In choice assays of the same accessions, there were no instances where apple maggot adults expressed a significant preference for a Marshall MacIntosh control when paired with any *Malus* accession. In fact, there were three instances where flies exhibited significant oviposition preference for fruit from *Malus* hybrid selections over a Marshall MacIntosh control ('E31-10' in the mid-season assay and 'E14-32' and 'PRI 1312-6' in a near harvest assay) (Table 3).

Oviposition varied significantly among the *Malus* fruit evaluated in mid-season ($F = 26.33$; df = 12, 107; $P < 0.001$), late season ($F = 10.83$; df = 10, 98; $P < 0.001$), and near harvest ($F = 3.28$; df = 6, 63; $P = 0.0072$) no-choice assays conducted in 2006 (Table 4). The lowest levels of oviposition occurred on smaller crabapple accessions such as *M. hupehensis*, *M. tschonoskii* (mid-season only), *M. sikkimensis* (mid-season only), *M.* × *zumi*, *M. yunnanensis*, and *M. bhutanica*. Adults oviposited on all accessions even though there were significant differences among the group evaluated. When oviposition data were corrected for fruit size, significant differences were observed for the mid-season ($F = 5.49$; df = 12, 107; $P < 0.001$) and late season ($F = 3.59$; df = 10, 98; $P < 0.001$) assays. No differences were observed in the near harvest assay ($F = 1.45$; df = 6, 63; $P = 0.2104$) (Table 4). Apple maggot adults oviposited more in Marshall MacIntosh fruit when paired with fruit of Delicious and all crabapple accessions in all choice assays conducted in 2006 (except for *M. bhutanica*'Macrocarpa' in the near harvest assay) (Table 5). However, there were no such preferences observed for Marshall MacIntosh over *Malus* hybrid selections ('E36-7', 'E7-54', 'E29-56', and PRI 1312-6) in any of the choice oviposition assays. As was the case in 2005, adults favored fruit from PRI 1312-6 over fruit from Marshall MacIntosh in the late season choice assay. When oviposition data were corrected for fruit size, fruit from E29-56 also were favored over control fruit in the late season assay (Table 5).

Larval Survival, 2005–2006. Larval survival in fruit varied significantly for both mid-season ($F = 7.58$; df = 12, 25; $P = 0.0005$) and near harvest ($F = 10.66$; df = 5, 11; $P = 0.0060$) assessments conducted in 2005 (Table 6). No larvae survived in E36-7' on fruit collected and assessed at mid-season in 2005, whereas survival was moderate to high among a number of other *Malus* accessions. In 2006, larval survival varied significantly in mid-season ($F = 131.19$; df = 12, 25; $P < 0.001$), late season ($F = 114.27$; df = 10, 21; $P < 0.001$), and near harvest ($F = 202.85$; df = 6, 13; $P < 0.001$) assays (Table 7). In all assays survival on E36-7 and all crabapple accessions was significantly lower than survival in Marshall MacIntosh. Although larval survival was moderate to high in a number of accessions previously described as resistant, no larvae survived in fruit of E36-7 during mid-season or late season evaluations. Meanwhile, mid-season and late season survival in E7-54 was significantly higher than survival in Marshall MacIntosh (Table 7).

Harvest Assessment of Apple Maggot Damage in the Field, 2006. Apple maggot damage varied significantly ($F = 6.49$; df = 11, 25; $P < 0.001$) in the field during a harvest-time assessment conducted in 2006 (Table 8). No damage was found on fruit from a number of crabapple accessions and only 1% damage was observed on E36-7. E29-56, E7-47, E14-32, and PRI 1312-6 exhibited the highest levels of feeding damage and none were significantly different than Delicious (Table 8).

Discussion

The *Malus* hybrid selections E7-54, E29-56, PRI 1293-102, and PRI 1312-6 have been previously described as resistant to apple maggot (Goonewardene and Howard 1989, USDA 2006). However, our data indicate that all of these accessions were very suscep-

Table 4. Mean fruit diameter and mean ± SEM number of female feeding and oviposition scars on fruit collected during the mid-season (17 July), late season (31 August), and near harvest (14 September) after 48-h exposure to single fruit in a no-choice assay with various *Malus* accessions in the laboratory, 2006

Date[a]	Accession	n[b]	Avg. fruit diam. (mm)	Punctures per fruit[c]	Punctures per sq. mm of fruit surface area[c]
17 July	*M. hupehensis*	10	7.4	0.0 (0.00)a	0.000 (0.000)a
	M. tschonoskii	5	18.0	0.6 (0.42)ab	0.005 (0.005)a
	M. sikkimensis	5	10.7	1.2 (0.69)ab	0.017 (0.014)a
	M. × *zumi* Calocarpa	10	8.5	1.6 (1.60)ab	0.030 (0.030)a
	M. spectabilis	10	10.8	2.9 (1.22)b	0.044 (0.019)ab
	Marshall MacIntosh	10	49.7	35.1 (3.06)d	0.124 (0.020)bc
	M. yunnanensis Vilmorin	10	12.6	10.3 (7.67)c	0.127 (0.038)bc
	E36-7	10	42.8	36.3 (9.18)d	0.146 (0.037)c
	M. bhutanica Macrocarpa	10	20.1	19.0 (7.67)c	0.152 (0.059)cd
	Delicious	10	45.8	43.9 (6.84)d	0.166 (0.024)cd
	PRI 1312-6	10	44.7	46.0 (8.30)d	0.173 (0.032)cd
	E7-54	10	43.3	44.5 (7.03)d	0.182 (0.031)cd
	E29-56	10	42.0	55.1 (6.00)d	0.225 (0.024)d
31 Aug.	*M. hupehensis*	10	10.3	1.4 (0.67)a	0.023 (0.011)a
	M. yunnanensis Vilmorin	10	13.1	4.3 (2.03)ab	0.053 (0.026)ab
	E36-7	10	51.3	18.1 (3.87)cd	0.062 (0.013)ab
	Marshall MacIntosh	10	58.3	29.7 (5.38)de	0.091 (0.017)abc
	E7-54	9	49.1	25.7 (4.69)cd	0.093 (0.018)abc
	M. × *zumi* Calocarpa	10	9.8	6.3 (2.67)ab	0.104 (0.043)abcd
	Delicious	10	55.6	36.2 (6.76)de	0.114 (0.020)bcde
	M. bhutanica Macrocarpa	10	23.5	22.5 (5.18)cd	0.159 (0.037)cde
	PRI 1312-6	10	58.7	59.0 (6.72)e	0.166 (0.019)cde
	M. spectabilis	10	12.0	13.3 (4.78)bc	0.184 (0.066)de
	E29-56	10	47.5	57.0 (6.87)e	0.211 (0.025)e
14 Sept.	*M. hupehensis*	10	11.6	8.1 (2.90)a	0.118 (0.043)a
	M. × *zumi* Calocarpa	10	11.1	11.6 (3.32)a	0.168 (0.049)a
	M. yunnanensis Vilmorin	10	13.8	12.7 (2.93)ab	0.146 (0.033)a
	M. spectabilis	10	12.4	16.9 (4.87)ab	0.228 (0.065)a
	M. bhutanica Macrocarpa	10	26.2	19.5 (4.36)ab	0.120 (0.026)a
	Marshall MacIntosh	10	67.8	31.0 (6.93)bc	0.081 (0.017)a
	E36-7	10	58.2	37.4 (6.45)c	0.114 (0.020)a

[a] Date of fruit harvest.
[b] Number of fruit examined.
[c] Means within each column and date grouping followed by the same letter are not significantly different ($P < 0.05$; Fisher's LSD).

Table 5. Mid-season (fruit picked 17 July), late season (31 August), and near harvest (fruit picked 14 September) adult oviposition preferences after 48 h of exposure, given a choice between a fruit from a given *Malus* accession and a Marshall MacIntosh control in the laboratory, 2006

Accession	n[a]	Mid-season preference[b]	Late season preference[b]	Near harvest preference[b]
Marshall MacIntosh	10	N	N	N
Delicious	10	C*	C	
E36-7	10	N	N	N
E7-54	10	N	N	
E29-56	10	N	N (A*)	
PRI 1312-6	10	N	A*	
M. hupehensis	10	C*	C*	C*
M. sikkimensis	5	C*		
M. spectabilis	10	C*	C*	C*
M. bhutanica Macrocarpa	10	C*	C*	N
M. tschonoskii	5	C*		
M. yunnanensis Vilmorin	10	C	C*	C*
M. × *zumi* Calocarpa	10	C*	C*	C

[a] Number of fruit pairs examined.
[b] N indicates no significant preference for either fruit, A indicates a significant preference for the experimental fruit, and C indicates a significant preference for the fruit of a Marshall MacIntosh control, as determined by a paired t-test ($P < 0.05$) with ln-transformed data.
*Indicates the preference was also significant when puncture data were adjusted for fruit size.

tible to adult oviposition and were very suitable for survival of apple maggot larvae inside fruit. Larval survival rates exceeded 77% in fruit from PRI 1312- and E7-54 during near harvest assessments conducted in 2005. Also in 2005–2006, 13–23 larvae per fruit emerged from fruit of the same accessions in both late season and near harvest assessments. Therefore, we conclude that these disease-resistant apple selections are not resistant to apple maggot.

Many of these earlier published conclusions (Goonewardene and Howard 1989, USDA 2006) were based upon limited data from unrepeated (or in some cases undocumented) assessments in the laboratory and the greenhouse field with insects of unknown origin. Criteria used to classify fruit as pest-resistant in these studies were not strong. In many cases, all that was required for a fruit to be labeled "resistant" was showing of less damage than a comparable control, even if damage levels on both cultivars indicated marked susceptibility. For example, three selections were categorized as codling moth resistant simply based on one stated significant statistical comparison with a 'Jonathan' control fruit, despite significant evidence of larval feeding in all fruit (Goonewardene 1987, Goonewardene and Howard 1989). Similarly, a number of these clones and/or selections described as

Table 6. Percentage of larval survival ± SEM from fruit of various *Malus* during mid-season (fruit picked 25 July) and near harvest (20 September) assays in 2005

Accession	n^b	Mean % larval survival, mid-season[c]	n^b	Mean % larval survival, near harvest[c]
E36-7	2	0.00 (0.00)a	2	29.30 (1.42)ab
M. sieversii GMAL 4304.e	2	11.22 (7.53)b		
E14-32	2	21.53 (3.09)bc	2	54.20 (5.98)abc
E7-47	2	31.23 (4.56)bc		
Marshall MacIntosh	2	31.23 (4.56)bcd	2	49.50 (0.95)abc
PRI 1312-6	2	32.39 (1.39)bcd	2	89.20 (10.78)c
PRI 1293-102	2	34.31 (19.11)bcd		
E29-56	2	34.37 (3.13)bcd	2	13.80 (2.38)a
E31-10	2	42.37 (7.63)bcde		
Delicious	2	46.65 (8.62)cde		
Liberty	2	46.72 (16.91)cde		
E7-54	2	53.50 (5.73)de	2	77.70 (3.96)bc
E11-24	2	60.97 (3.92)e		

[a] Date of fruit harvest.
[b] Number of five fruit cohorts examined for survival determination.
[c] Means within each column followed by the same letter are not significantly different ($P < 0.05$; Fisher's LSD).

Table 7. Percentage of larval survival from fruit of various *Malus* during mid-season (fruit picked 17 July), late season (31 August), and near harvest (fruit picked 14 September) assays in 2006

Date[a]	Accession	n^b	Mean % larval survival, (± SEM)[c]
17 July (mid-season)	*M. hupehensis*	2	0.00 (0.00)a
	M. sikkimensis	1	0.00a
	M. spectabilis	2	0.00 (0.00)a
	M. bhutanica Macrocarpa	2	0.00 (0.00)a
	M. tschonoskii	1	0.00a
	M. × *zumi* Calocarpa	2	0.00 (0.00)a
	M. yunnanensis Vilmorin	2	0.00 (0.00)a
	E36-7	2	0.00 (0.00)a
	Delicious	2	4.00 (0.43)b
	PRI 1312-6	2	5.36 (1.41)b
	E29-56	2	11.62 (0.43)c
	Marshall MacIntosh	2	32.18 (5.82)d
	E7-54	2	42.01 (3.19)e
31 Aug. (late season)	*M. hupehensis*	2	0.00 (0.00)a
	M. spectabilis	2	0.00 (0.00)a
	M. bhutanica Macrocarpa	2	0.00 (0.00)a
	M. × *zumi* Calocarpa	2	0.00 (0.00)a
	M. yunnanensis Vilmorin	2	0.00 (0.00)a
	E36-7	2	0.00 (0.00)a
	Delicious	2	26.31 (4.03)b
	E29-56	2	32.10 (7.28)b
	PRI 1312-6	2	34.17 (2.15)b
	Marshall MacIntosh	2	45.88 (6.64)c
	E7-54	2	64.18 (0.82)d
14 Sept. (near harvest)	*M. hupehensis*	2	0.00 (0.00)a
	M. spectabilis	2	0.00 (0.00)a
	M. bhutanica Macrocarpa	2	0.00 (0.00)a
	M. × *zumi* Calocarpa	2	0.00 (0.00)a
	M. yunnanensis Vilmorin	2	0.00 (0.00)a
	E36-7	2	4.25 (1.54)b
	Marshall MacIntosh	2	52.28 (4.06)c

[a] Date of fruit harvest.
[b] Number of five fruit cohorts examined for survival determination.
[c] Means within each column and date grouping followed by the same letter are not significantly different ($P < 0.05$; Fisher's LSD).

"resistant" had fruit that were very susceptible to oviposition damage by apple maggot adults, and larvae were able to complete development successfully in fruit from all but one of the releases (Goonewardene et al. 1975, 1979; Goonewardene and Howard 1989).

Numerous clarifications and revisions need to be made to accession annotations on the USDA–GRIN database to accurately describe their true resistance status with regard to various direct feeding apple insect pests. Beyond the accessions evaluated here, there are seven additional *Malus* hybrid selections currently listed on GRIN with claims of apple maggot resistance (USDA 2006). These selections, which are currently housed in PGRU collections outside of the core collection, should be reevaluated in rigorous laboratory studies to determine whether they are truly resistant to apple maggot oviposition, subsequent survival of larvae in the fruit, or both.

Our findings were consistent with earlier published observations of apple maggot response to a number of crabapple accessions. Although we observed statisti-

Table 8. Mean percentage ± SEM of fruit damaged by apple maggot in the field on various *Malus* accessions housed at the USDA–PGRU core collection in Geneva, NY, during a harvest (3–6 October) assessment, 2006

Accession	n^a	% damaged fruit (± SEM)[b]
M. hupehensis	3	0.00 (0.00)a
M. spectabilis	4	0.00 (0.00)a
M. bhutanica Macrocarpa	2	0.00 (0.00)ab
M. × *zumi* Calocarpa	3	0.00 (0.00)ab
M. yunnanensis Vilmorin	2	0.00 (0.00)ab
E36-7	2	1.00 (1.00)abc
Delicious	4	18.75 (8.19)bcd
Liberty	4	26.00 (13.88)cd
E29-56	3	30.67 (8.35)cd
E7-47	3	37.33(11.79)de
E14-32	3	42.00 (16.65)de
PRI 1312-6	4	66.00 (15.34)e

[a] Number of tree replicates examined.
[b] Means within each column followed by the same letter are not significantly different ($P < 0.05$; Fisher's LSD).

Table 9. Typical harvest dates of a number of disease-resistant *Malus* hybrid selections, as listed on the USDA–GRIN, 2006

Accession	Typical harvest period in New York[a]		
M. hybrid E29-56	Medium/early	5–15 Sept.	20–30 d before Delicious
M. hybrid E7-47	Medium/early	5–15 Sept.	20–30 d before Delicious
M. hybrid E7-54	Medium	25 Sept.	10 d before Delicious
M. hybrid E36-7	Late	15 Oct.	10 d after Delicious
M. hybrid E11-24	Early	5–25 Sept.	30–50 d before Delicious
M. hybrid E31-10	Early	5–25 Sept.	30–50 d before Delicious
M. hybrid E14-32	Very early	26 Aug.–5 Sept.	50–60 d before Delicious
M. hybrid PRI 1312-6	Medium/late	5 Oct.	Same as Delicious
M. hybrid PRI 1293-102	Late	15 Oct.	10 d after Delicious
Marshall MacIntosh	Medium	25 Sept.	10 d before Delicious

[a] Data taken from GRIN online database (USDA 2006).

cal differences in the level of oviposition between crabapple species and larger apple fruit, adults were able to lay eggs on fruit from all *Malus* accessions observed. Similarly, Neilson (1967) and Pree (1977) reported successful oviposition damage on all *Malus* species evaluated, including *M. sikkimensis* and *M. bhutanica* (formerly *M. toringoides*), which also were evaluated in this study. Reissig et al. (1990) reported that oviposition damage from apple maggot varied significantly among fruit from 25 crab apple species evaluated in both the field and in multiple choice laboratory oviposition assays. Oviposition was lower on a number of smaller crabapple species, including *M. sikkimensis*, *M.* × *zumi* Calocarpa, *M. yunnanensis* Vilmorin, and *M. hupehensis* under multiple-choice conditions. Based on these observations and our evaluations under no-choice and choice conditions, we conclude that these species are less preferred by apple maggot adults, but are readily accepted by gravid females when no other fruit are available.

Larval survival varied significantly across *Malus* species, with no larval survival observed on any of the crabapple species evaluated. Two of the species tested in this study, *M. spectabilis*, and *M. tschonoskii* have not been previously reported to be resistant to survival of apple maggot larvae. Our results are consistent with earlier studies that found zero larval survival in fruit from *M. sikkimensis* and *M. bhutanica* (Neilson 1967, Pree 1977). In laboratory tests reported by Reissig et al. (1990), females oviposited and eggs hatched in all 25 crabapples evaluated but larvae did not survive in *M. hupehensis* or *M* × *zumi* Calocarpa. Larval mortality was very high in fruit from *M. yunnanensis* Vilmorin and *M. sikkemensis* (Hood.) Koehne.

Although susceptible to adult oviposition, fruit from E36-7 seem to be quite resistant to larval feeding damage during the summer, when apple maggot adults are most likely to oviposit in the developing fruit. No larvae survived in fruit of this selection during a late August assay in 2006 or in mid-season assays conducted in either 2005 or 2006. Our laboratory data indicate that the only period of susceptibility to larval feeding seems to come as the fruit approaches harvest, in late September and early October. This is in stark contrast to related disease-resistant accessions such as E14-32 and E29-56, both of which share a common parent with E36-7, but both of which also were very susceptible to larval feeding by apple maggot. Ironically, E36-7 was originally reported to have resistance to attack from the coleopteran pest, plum curculio, *Conotrachelus nenuphar* (Herbst) (Goonewardene 1987), but these reports also have been shown to be erroneous (Myers et al. 2007). This is the first report of confirmed resistance to larval feeding from apple maggot in any commercial apple selection or cultivar.

It is often difficult to determine whether differences in survival of apple maggot larvae in infested fruit of different types of germplasm are due to seasonal differences in fruit texture, fruit size, or phytochemical components. Previous studies have noted that fruit maturity or firmness can affect larval survival, because larvae are generally observed to have lower rates of survival in hard-fleshed, or late-maturing varieties of apple (Dean and Chapman 1973, Reissig et al. 1990). Also, apple maggot larval survival is influenced by the maturity of fruit when it is infested, and whether apples remain on the tree. For example, Reissig (1979) found that apple maggot eggs hatched and larvae completed development in different maturities of apples that were picked from the tree and incubated in the laboratory but that larval mortality was high in apples remaining on the tree, particularly in fruit infested in late June and early July. Presumably, developing larvae in apples that remain on the tree and are infested relatively early in the season may be killed by internal force within the rapidly expanding fruit. However, because the laboratory tests in this study were conducted with fruit that was infested after it was removed from the tree, observed survival differences were not due to fruit expansion.

Survival of larvae in fruit tested in this study was not necessarily related to maturation dates. Indeed, E36-7 is a late-maturing variety with a listed harvest date of 10 d after Delicious (Table 9), and larvae were unable to survive in fruit infested at various times of the season until very late in the season. However, PRI 1293-12 is also a late-maturing variety (USDA 2006), but it was very suitable for survival of apple maggot larvae even when it was infested relatively early in the season. Therefore, it is likely that factors other than fruit maturity are responsible for the observed resistance to larval feeding in E36-7.

It is possible that the relatively small size of crabapples could have an effect on the survival of

apple maggot larvae, because most of the selections in which larvae did not survive are smaller than most types of commercial apple cultivars or selections. The only observed exception to this relationship was the low survival of larvae in the fruit E36-7 that was of comparable size to the other *Malus* × *domestica* cultivars and hybrid selections observed. Additionally, subsequent regression analyses indicated that adult oviposition was positively correlated with fruit diameter for the mid-season and late season no-choice oviposition assays conducted in 2006. Beyond size, oviposition on fruit may have been affected by other visual cues, or by variability in fruit volatiles, which have been shown to affect host choice of apple maggot adults.

It is likely that in addition to fruit size and maturity, fruit phytochemistry can affect apple maggot larval survival and development. Pree (1977) showed that crabapple resistance was correlated with total phenol content and further demonstrated that addition of 1,000 ppm of phenolic acids, gallic, tannic, and *o*-coumaric acids, quercetin, naringen, and *d*-catchin to an artificial laboratory diet prevented larval development. We hypothesize that further evaluations of fruit phytochemistry in apple might reveal important genetically based variations that are important to apple maggot larval survival and development. Bioactive phenolic compounds, which are known to have key effects on insect survival and development, for example, have been shown to vary significantly in their occurrence and concentrations in a number of apple tissues and extracts (Treutter 2001) and across apple cultivars (Marks et al. 2007). Variation in expression of phenolic compounds in various *Malus* germplasm would be an important area to investigate as researchers seek to understand the underlying mechanisms of host plant resistance to apple maggot and other fruit feeding pest species.

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